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PRESYNAPTIC AND POSTSYNAPTIC DEPRESSANT EFFECTS OF PHENYTOIN SODIUM AT THE NEUROMUSCULAR JUNCTION

P.W. GAGE, M. LONERGAN & T.A. TORDA

School of Physiology and Pharmacology, University of New South Wales, Kensington, 2033, Australia

1 Phenytoin sodium, 10 μg/ml (3.6×10^{-5} M), reduces the amplitude of endplate potentials in mouse sternomastoid neuromuscular junctions.

2 The reduction in amplitude is due to a reduction both in the quantal content of endplate potentials and in the amplitude of the voltage response to quanta of acetylcholine.

3 The reduction caused by phenytoin in the amplitude of spontaneous miniature end plate potentials was due to a reduction in the time constant of decay of miniature endplate currents.

4 It is concluded that phenytoin depresses neuromuscular transmission by reducing both the amount of acetylcholine secreted in response to an action potential and by reducing the lifetime of postsynaptic channels activated by acetylcholine.

Introduction

Phenytoin sodium is a drug that has been in clinical use as an anticonvulsant for nearly 40 years (Merritt & Putnam, 1938). More recently a cardiac antiarrhythmic effect similar to that of local anaesthetics has been discovered and put to clinical use (Mercer & Osborne, 1967; Moe & Abildskov, 1975). The combination of anticonvulsant and antiarrhythmic actions aroused our interest in this drug. In its central depressant action it resembles barbiturates which have been shown to reduce the time constant of decay of endplate currents (e.p.cs) (Adams, 1974; 1976; Seyama & Narahashi, 1975; Torda & Gage, 1976) and to increase the quantal content of end plate potentials (e.p.ps) (Thompson & Turkanis, 1973). In its cardiac antiarrhythmic effect, it resembles lignocaine which also affects the time course of endplate currents (Steinbach, 1968; Ruff, 1976). We have therefore investigated the effect of phenytoin sodium on synaptic transmission at the mammalian endplate.

Methods

Two types of experiments were performed. In the first, the effect of phenytoin sodium on the average quantal content of e.p.ps was examined; in the second its effect on miniature end plate currents (m.e.p.cs) was determined. As the therapeutic range (blood concentration) for the anticonvulsant effects of this drug is 10 to 30 μg/ml and it is 70 to 95% protein bound (Woodbury & Fingl, 1975), the concentration used here was 10 μg/ml (3.6×10^{-5} M).

An excised mouse sternomastoid muscle or diaphragm was pinned out in a tissue bath of 3 ml capacity and superfused with control solution containing (mmol/l): NaCl 120, $NaHCO_3$ 25, KCl 3.5, $CaCl_2$ 2.5, $MgCl_2$ 1 and glucose 11. Solutions were bubbled continuously with 5% CO_2 in O_2 and the perfusion rate was 2 ml/min. The pH of the solution was 7.40 to 7.45 and the temperature of the tissue bath was maintained at 27.0 ± 0.5°C. Phenytoin sodium (Dilantin, Parke Davis) was dissolved in 40% propylene glycol and 10% ethanol in water to make a 50 mg/ml drug concentrate and the pH adjusted to 11 with NaOH; 0.2 ml of the drug concentrate was added to 1 litre of solution for these experiments and a similar volume of solvent was added to control solution for comparison.

The effect of phenytoin sodium on the quantal content of e.p.ps was investigated in excised mouse sternomastoid nerve-muscle preparations. The perfusing solution was modified by increasung the $MgCl_2$ concentration to 12 mmol/l in order to reduce mean quantal content and the NaCl concentration was reduced by 18 mmol/l. The nerve was stimulated supramaximally at 0.5 Hz. E.p.ps were recorded with electrodes filled with 3 M KCl (resistance 10 to 15 megohm). Quantal content (1.9 to 25) was calculated from the coefficient of variation of 100 to 120 e.p.ps (Del Castillo & Katz, 1954). In the last two experiments of Table 1, quantal content was calculated from the ratio of mean e.p.p. to mean m.e.p.p. amplitude

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(n = 64 and 128). Only cells in which the membrane potential was between −70 and −80 mV were used. No correction was made for non-linear summation: e.p.ps were generally less than 10 mV in amplitude.

M.e.p.cs were recorded from mouse diaphragms through extracellular electrodes filled with 1 M NaCl in agar (resistance 0.5 to 1 megohm). A transient recorder (Neurograph N-3, Transidyne General Corp.) was used to 'capture' the currents. The data from the transient recorder were processed by computer (PDP8/E, Digital Equipment Corporation) programmed to calculate the time constant of decay from a least squares fit to points sampled at 10 μs intervals during the decay. The peak amplitude and growth time (20 to 80% of peak amplitude) of the m.e.p.cs were also measured by computer.

Results

Effect of phenytoin on endplate potentials

On exposure to a solution containing phenytoin sodium (10 μg/ml) the mean amplitude of e.p.ps was reduced. There was no significant change in membrane potential. Reductions in e.p.p. amplitude were seen in five experiments. This could have been due to a reduction in the number of quanta of acetylcholine secreted in response to an action potential or to a reduction in the postsynaptic voltage response to a quantum of acetylcholine. In order to test these possibilities, the quantal content of e.p.ps was measured before and after exposure to phenytoin sodium and was found to be reduced by the drug (Table 1). It was also found that there was a reduction in the mean amplitude of m.e.p.ps, but an even greater reduction in the mean amplitude of e.p.ps confirmed that the drug caused a reduction in the mean quantal content of e.p.ps. Results obtained in five experiments are given in Table 1. The effect could be completely reversed by washing out the drug.

It was noticed that it was sometimes necessary to increase the strength of the stimulus to the nerve after exposure to the phenytoin. This observation was not quantitatively studied.

Table 1 The effect of phenytoin sodium (10 μg/ml) on the quantal content of endplate potentials (e.p.ps)

	Quantal content	
Experiment	*Control*	*Phenytoin*
1	25.0	8.9
2	9.7	2.7
3	6.4	1.9
4	2.7	1.5
5	1.0	0.6

Effect of phenytoin on miniature endplate currents

The reduction in m.e.p.p. amplitude could have been due to a reduction in the amplitude of the underlying m.e.p.cs or to an increase in their rate of decay (Gage, 1976). To evaluate these possibilities, the effect of phenytoin on the time course of m.e.p.cs was examined. The decay of m.e.p.cs remained exponential in the phenytoin. It was found that the concentration of phenytoin sodium tested significantly reduced the time constant of decay of m.e.p.cs but had little effect on their amplitude. For example, in one experiment the time constant of decay of 72 m.e.p.cs in control solution was 1.17 ± 0.08 ms (mean ± s.e. mean). After exposure to phenytoin sodium for 20 min the time constant of decay was reduced to 0.82 ± 0.05 ms. This reduction was statistically significant ($P < 0.01$, Student's t test). Similar results were obtained in three experiments and details are given in Table 2.

In addition to the reduction in the time constant of decay of m.e.p.cs there appeared to be a reduction in the growth time of m.e.p.cs in the presence of the phenytoin. In the three experiments, the growth time fell from 175 ± 19 ms in control solution to 146 ± 10 ms in the solution containing phenytoin. Although this is not statistically significant, the effect probably warrants further study.

Discussion

The results show that phenytoin has two types of depressant action at the neuromuscular junction. Firstly, it reduces the time constant of decay of m.e.p.cs and this causes a reduction in the amplitude of m.e.p.ps (Gage, 1976). Secondly, it causes a decrease in the average number of quanta of acetylcho-

Table 2 The effect of phenytoin sodium (10 μg/ml) on the time constant of decay of miniature end plate currents (mean ± s.e. mean) recorded in three experiments

	Time constant (ms)		
Experiment	*Control*	*Phenytoin*	%
1	1.22 ± 0.08	1.07 ± 0.08	86
2	1.08 ± 0.07	0.93 ± 0.05	86
3	1.17 ± 0.08	0.82 ± 0.05	70
	1.16 ± 0.04	0.94 ± 0.04*	81

Grand means are given in the bottom line.
* $P < 0.01$.

line released in response to an action potential. Depression of evoked transmitter release in the presence of phenytoin at frog neuromuscular junctions has been noted previously (Yaari, Pincus & Argov, 1977; 1979). Depression of transmitter-release was obtained with normal extracellular calcium concentration, as found here. The greater depression they observed may be related to the higher concentrations of phenytoin used. Yaari *et al.* (1979) postulated that phenytoin depressed calcium influx across the presynaptic membrane and this may indeed underlie the observed presynaptic effect.

The postsynaptic effect is interesting because a wide variety of general anaesthetics have a similar effect at the neuromuscular junction, and it has been suggested that their synaptic depressant effect in the central nervous system may be mediated in the same way (Gage & Hamill, 1975; Torda & Gage, 1976; 1977). A reduction in decay time constant of m.e.p.cs generally reflects a decrease in the average lifetime of endplate channels (Anderson & Stevens, 1973). If phenytoin reduces postsynaptic channel lifetime at central synapses, synaptic transmission would be depressed and this may underline its anticonvulsant action. On the other hand, a reduction in transmitter secretion may be a more significant central effect.

Phenytoin has been reported to depress preferentially polysynaptic reflexes (Esplin, 1957) and to depress post-tetanic potentiation and repetitive activity (Riker, Werner, Roberts & Kuperman, 1959). These actions may be related to the pre- and postsynaptic depressant effects we have observed at the neuromuscular junction.

References

ADAMS, P.R. (1974). The mechanism by which amyolbarbitone and thiopentone block the end-plate response to nicotinic agonists. *J. Physiol.*, **241**, 41–42.

ADAMS, P.R. (1976). Drug blockage of open end-plate channels. *J. Physiol.*, **260**, 531–552.

ANDERSON, C.R. & STEVENS, C.F. (1973). Voltage clamp analysis of acetylcholine produced end-plate current fluctuations at frog neuromuscular junction. *J. Physiol.*, **235**, 655–91.

DEL CASTILLO, J. & KATZ, B. (1954). Quantal components of the end-plate potential. *J. Physiol.*, **124**, 560–573.

ESPLIN, D.W. (1957). Effects of diphenylhydantoin on synaptic transmission in cat spinal cord and stellate ganglion. *J. Pharmac. exp. Ther.*, **120**, 301–23.

GAGE, P.W. & HAMILL, O.P. (1975). General Anesthetics: Synaptic depression consistent with increased membrane fluidity. *Neurosci. Letts*, **1**, 61–65.

GAGE, P.W. (1976). Generation of end-plate potentials. *Physiol. Rev.* **56**, 177–247.

MERCER, E.N. & OSBORNE, J.A. (1967). The current status of diphenylhydantoin in heart disease. *Ann. intern. Med.*, **67**, 1084–1107.

MERRITT, H.H. & PUTNAM, T.J. (1938). Sodium diphenylhydantoinate in treatment of convulsive disorders. J. Am. med. Assoc., **111**, 1608–73.

MOE, G.K. & ABILDSKOV, J.A. (1975). Antiarrhythmic drugs. *The Pharmacological Basis of Therapeutics.* ed Goodman, L.S. & Gilman, A. 5th ed. pp. 698. New York: Macmillan Co. Inc.

RIKER, W.F., WERNER, G., ROBERTS, J. & KUPERMAN, A. (1959). Pharmacological evidence for the existence of a presynaptic event in neuromuscular transmission. *J. Pharmac. exp. Ther.*, **125**, 150–158.

RUFF, R.L. (1976). Local anesthetic alteration of miniature end-plate currents and end-plate current fluctuations. *Biophys. J.*, **16**, 433–439.

SEYAMA, I. & NARAHASHI, T. (1975). Mechanism of blockade of neuromuscular transmission by pentobarbital. *J. Pharmac. exp. Ther.*, **192**, 95–104.

STEINBACH, A.B. (1968). Alteration by xylocaine (lidocaine) and its derivatives of the time course of the end-plate potential. *J. gen. Physiol.*, **52**, 144–161.

THOMSON, T.D. & TURKANIS, S.A. (1973). Barbiturate-induced transmitter release at a frog neuromuscular junction. *Br. J. Pharmac.*, **48**, 48–58.

TORDA, T.A. & GAGE, P.W. (1976). Effect of barbiturates on synaptic currents. *Anaesthesia and Intensive Care*, **4**, 199–202.

TORDA, T.A. & GAGE, P.W. (1977). Postsynaptic effect of I.V. anesthetic agents at the neuromuscular junction. *Br. J. Pharmac.*, **49**, 771–776.

WOODBURY, D.M. & FINGL, E. (1975). In *The Pharmacological Basis of Therapeutics.* ed. Goodman, L.S. & Gilman, A. 5th edition, p. 206. MacMillan Co: New York.

YAARI, Y., PINCUS, J.H. & AGROV, Z. (1977). Depression of synaptic transmission by diphenylhydantoin. *Ann. Neurol.*, **1**, 334–338.

YAARI, Y., PINCUS, J.H. & ARGOV, Z. (1979.) Phenytoin and transmitter release at the neuromuscular junction of the frog. *Brain Res.*, **160**, 479–487.

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